

SUPPLEMENTAL AMENDMENT UNDER 37 CFR § 1.111
Serial Number: 09/834095
Filing Date: April 12, 2001
Title: VIRUSES COMPRISING MUTANT ION CHANNEL PROTEIN

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In the Claims

Please amend the claims as follows:

1. (Currently Amended) An isolated and purified recombinant influenza virus comprising a mutant M2 ion channel protein which lacks or has reduced activity relative to the corresponding wild-type M2 ion channel protein, wherein the mutation is in the transmembrane domain of the M2 ion channel protein, wherein the mutation does not substantially alter the *in vitro* replication of the virus in the absence of amantadine but is associated with attenuation of the virus *in vivo*, and wherein the mutant M2 ion channel protein lacks one or more residues in the transmembrane domain which include residues corresponding to residues 29 to 31 of the M2 protein.
- 2-8. (Canceled)
9. (Original) The isolated and purified virus of claim 1 wherein the recombinant virus further comprises a heterologous immunogenic protein of a pathogen.
10. (Original) A vaccine comprising the isolated and purified virus of claim 1.
- 11-17. (Canceled)
18. (Original) A method to immunize a vertebrate, comprising: contacting the vertebrate with an effective amount of the recombinant virus of claim 1.
19. (Original) The method of claim 18 wherein the vertebrate is an avian.
20. (Original) The method of claim 18 wherein the vertebrate is a mammal.
21. (Original) The method of claim 18 wherein the vertebrate is a human.

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22. (Currently Amended) A composition comprising a plurality of influenza vectors, comprising:

a) at least two vectors selected from a vector comprising a promoter operably linked to an influenza virus PA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB1 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB2 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus HA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NP cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus M cDNA linked to a transcription termination sequence, and a vector comprising a promoter operably linked to an influenza virus NS cDNA linked to a transcription termination sequence, wherein the M cDNA comprises a mutant M2 ion channel protein DNA comprising a mutation in the transmembrane domain of the M2 ion channel protein, wherein the mutant M2 ion channel protein lacks or has reduced activity relative to the corresponding wild-type M2 ion channel protein, wherein the mutation does not substantially alter the *in vitro* replication of a virus having the mutant M2 ion channel protein in the absence of amantadine but is associated with attenuation of the virus *in vivo*, and wherein the mutant M2 ion channel protein lacks one or more residues in the transmembrane domain which include residues 29 to 31; and

b) at least two vectors selected from a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB1, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB2, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NP, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus HA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus M1, a vector comprising a promoter operably linked to a DNA segment encoding an ion channel protein, and a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NS2.

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23. (Original) The composition of claim 22 further comprising a vector comprising a promoter operably linked to a DNA fragment of interest in antisense orientation.
24. (Original) The composition of claim 23 wherein the vector comprises a DNA fragment which encodes an immunogenic polypeptide or peptide of a pathogen.
25. (Currently Amended) An isolated virus prepared by contacting a host cell with a plurality of influenza vectors, wherein the plurality of vectors comprises: a) at least two vectors selected from a vector comprising a promoter operably linked to an influenza virus PA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB1 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB2 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus HA cDNA linked to a transcription termination sequence, a vector comprising promoter operably linked to an influenza virus NP cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus M cDNA linked to a transcription termination sequence, and a vector comprising a promoter operably linked to an influenza virus NS cDNA linked to a transcription termination sequence, wherein the M cDNA comprises mutant M2 ion channel protein DNA comprising a mutation in the transmembrane domain, wherein the mutant M2 ion channel protein lacks or has reduced activity relative to the corresponding wild-type M2 ion channel protein, which wherein the mutation does not substantially alter the *in vitro* replication of the virus in the absence of amantadine but is associated with attenuation of the virus *in vivo*, and wherein the mutant M2 ion channel protein lacks one or more residues in the transmembrane domain which include residues corresponding to residues 29 to 31 of the M2 protein; and b) at least two vectors selected from a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB1, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB2, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NP,

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a vector comprising a promoter operably linked to a DNA segment encoding influenza virus HA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus M1, a vector comprising a promoter operably linked to a DNA segment encoding an ion channel protein, and a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NS2.

26. (Original) A host cell contacted with the virus of claim 1 or 25.

27-30. (Canceled)

31. (Previously Presented) The isolated and purified virus of claim 1 wherein the mutation is the deletion of residues 29 to 31 of the transmembrane domain of M2.

32. (Currently Amended) The isolated and purified virus of claim 1 wherein the mutation provides a selective growth advantage to the recombinant virus in the presence of a concentration of amantadine which inhibits the replication of a corresponding virus which does not comprise a the mutant M2 ion channel protein.

33. (Currently Amended) A method of preparing a recombinant influenza virus comprising a mutant ion channel protein which lacks or has reduced activity relative to the corresponding wild-type ion channel protein, comprising:

(i) contacting a host cell with a plurality of influenza vectors so as to yield recombinant influenza virus, wherein the plurality of vectors comprises: a) at least two vectors selected from a vector comprising a promoter operably linked to an influenza virus PA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB1 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB2 cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus HA cDNA linked to a transcription termination sequence, a vector comprising promoter operably linked to an

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to a transcription termination sequence, a vector comprising promoter operably linked to an influenza virus NP cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NA cDNA linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus M cDNA linked to a transcription termination sequence, and a vector comprising a promoter operably linked to an influenza virus NS cDNA linked to a transcription termination sequence, wherein the M cDNA comprises mutant M2 ion channel protein DNA which encodes a mutant M2 ion channel protein which lacks or has reduced activity relative to the corresponding wild-type M2 ion channel protein, wherein the mutation is in the transmembrane domain of the M2 ion channel protein, wherein the mutation does not substantially alter the *in vitro* replication of the virus in the absence of amantadine but is associated with attenuation of the virus *in vivo*, and wherein the mutant M2 ion channel protein ~~lacks~~ ~~lack~~ one or more residues in the transmembrane domain which include residues ~~corresponding to residues~~ 29 to 31 of the M2 protein; and b) at least two vectors selected from a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB1, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus PB2, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NP, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus HA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NA, a vector comprising a promoter operably linked to a DNA segment encoding influenza virus M1, a vector comprising a promoter operably linked to a DNA segment encoding an ion channel protein, and a vector comprising a promoter operably linked to a DNA segment encoding influenza virus NS2; and

(ii) isolating the virus.

34. (Previously Presented) The method of claim 33 wherein the mutation is the deletion of residues 29 to 31 of the transmembrane domain of M2.

35. (Previously Presented) Virus prepared by the method of claim 33.